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Genetic-Environmental Interactions in Relation to Low Dose Studies: A Possible Model from Breast Cancer

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The identification of genetically susceptible individuals may permit the determination of genetic and environmental interactions which result in disease. This paper presents some recent findings on possible genetic-environmental interactions in breast disease as determined by a "nature-nurture" model employing epidemiological risk factors and cytologic studies of breast secretions obtained by nipple aspiration. The findings indicate that severe changes in the cytologic characteristics of the fluid are associated with a positive family history of breast cancer and clinical fibrocystic disease. These findings were interpreted as supporting the hypothesis that women with such a family history may have increased susceptibility to environmental factors. This model may have utility in other environmental epidemiologic studies.

An important objective of research in environmental epidemiology is to detect those individuals who, because of their genetic susceptibility to environmental factors, are prone to develop illness. Identifying genetic susceptibility may permit us to determine how environmental factors interact to produce disease and so lead to the development of preventive measures.

Genetic-environmental interactions have been recognized since the early days of genetics. But not until the recent development of biochemical genetics and pharmacogenetics has the potential influence of differential genetic susceptibility in host response to varying environments been appreciated. The studies indicate that genetically determined differences in an individual's constitution may determine how that person will respond to environmental chemical agents (*1*).

More recently, it has been found that, in addition to ionizing radiations, many chemical agents can damage DNA, alter its biochemical and physiologic expression and, in some individuals, lead to ill health and disease (*2*). The problem is compounded because most known environmental agent factors implicated in the major noninfectious diseases are weak; the diseases allegedly caused by them involve low dose rates as well as the interaction of multiple factors.

An early example of genetic-environmental interaction related to disease is differential susceptibility to malaria of children who have or do not have the sickle trait (*3*). Other examples include G6PD deficiency—an X-linked trait—and susceptibility to primaquin, and, as found later, differing susceptibility to a host of chemical substances (*4*). The abnormal response of erythrocytes in G6PD deficiency to numerous drugs stimulated the development of the field of pharmacogenetics (*5*). A variety of genetic systems have been identified through the use of and reaction to medications. A recent example, and a model for studies in the metabolism of carcinogens, revealed genetic differences among individuals in metabolizing the antituberculosis drug isoniazid by liver acetyltransferase. Subsequent studies indicated considerable genetic-racial variation in the inactivation of the drug.

Through the pioneering work of the Millers and many subsequent workers, it is now evident that most procarcinogens are metabolically activated

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by cellular enzymes to the ultimate chemical carcinogenic form (*6*). The process involves the microsomal cytochrome P448-450 enzyme systems which detoxify and metabolize many toxic substances before their elimination. The liver is the major site of these enzymes, but all tissues, including the breast, show some enzymatic activity. Experiments in mice indicate that the metabolism of polycyclic hydrocarbons is under genetic control and that different mouse strains have differing metabolism and susceptibility to carcinogenesis for these substances (*7*). Kellerman, Shaw and others (*8, 9*) reported that exposure of human lymphocyte cultures to benzpyrene led to the induction of BP-hydroxylase enzyme and that lymphocytes from lung cancer patients could be induced to higher enzyme levels than lymphocytes from controls. It was suggested that the mechanism might underlie the differential susceptibility to lung cancer in response to cigarette smoke, known to contain benzpyrene and related polycyclic hydrocarbons. The earlier belief that inducibility of BP-hydroxylase was inherited as a simple Mendelian trait giving three distinct genotypes was not confirmed by other researchers. Instead, it appears that there is continuous variation from low to high levels of BP-hydroxylase inducibility among members of the population, indicating a more complex genetic variation in BP-hydroxylase inducibility.

In response to the DNA-damaging action of environmental chemical agents, nature has evolved enzyme systems for DNA repair. The repair mechanisms came to light after Cleaver reported that skin cells from xeroderma pigmentosum, a genetically dominant disease leading to skin cancer, were unable to repair their DNA after damage by ultraviolet light, x-rays and carcinogenic chemicals (*10*). Similar defective DNA-repair systems were demonstrated in other rare genetic conditions such as ataxia telangiectasia and in Fanconi's anemia (*11*). However, in ordinary cancers, the genetic predisposition probably resides in the differing metabolic rates and the activation of mutagens to carcinogens that tend to overwhelm the normal DNA-repair mechanisms.

Recent work in our laboratory, which deals with the genetic epidemiology of breast cancer, indicates possible genetic-environmental interaction. That breast cancer in some women had a familial component has been known for many years (*12*). Women with a first degree relative (mother, sister, daughter) with breast cancer have a two-to threefold increased risk of also developing breast cancer. The risk is increased significantly if the cancer in the relative occurred premenopausally and was bilateral. Very recently, King, Elston, Go, Lynch and Petrakis (*13*) reported evidence for the existence of an allele that increases susceptibility to human breast cancer in certain very high risk families. The susceptibility allele appears to be linked to the polymorphic glutamate-pyruvate transaminase (GPT) locus on chromosome 10. However, because the GPT genetic marker is not associated with risk of breast cancer in the general population, it cannot be used as a screening device for susceptibility to breast cancer. Nevertheless, the finding strengthens the likelihood that genetic susceptibility to breast cancer exists and that it might be detected by other techniques. This would enable the investigation of genetic interaction with "environmental" factors. The present study presents some new evidence from our research on nipple aspirates of breast fluid which may be indicative of potential genetic-environmental interactions involving breast epithelium.

Over the past seven years our staff has been investigating the genetic epidemiology and pathophysiology of breast cancer and benign breast disease, with emphasis on breast secretory activity. We have developed a working hypothesis that emphasizes breast epithelial metabolic and secretory activity as a key factor in the pathogenesis of breast disease (*14*). The breast epithelium contains the enzymatic mechanism for metabolizing potentially toxic and carcinogenic substances transported in the blood. These genetically conditioned metabolic and physiologic activities may, in the appropriate environmental circumstances, lead to epithelial damage and ultimately to breast disease. Before discussing our most recent cytologic results, some of our basic findings on nipple aspirates of breast fluids will be presented.

The women in this study had voluntarily come to be examined at the University of California Hopsitals and Clinics, the Breast Screening Center of Northern California, and other community health and screening centers. All women completed a questionnaire on their demographic, clinical, marital, reproductive and other characteristics. A specially trained nurse or a physician examined their breasts. On both breasts, nipple aspiration was attempted, employing the technique described previously (*15*). A woman was defined as "secretor" if a drop or more of fluid appeared at the nipple surface after application of the breast pump with the syringe retracted to 10 cc and held up to 15 sec. It is necessary to exert this degree of negative pressure to force breast fluid through the closed nipple ducts of most nonlactating adult women. We believe that applications of lower negative pressures, as reported by others, may not provide an accurate estimate of breast secretory activity. Our

findings will be presented in terms of (a) breast secretor status and breast cancer risk factors, (b) secretion of mutagenic substances and (c) use of breast fluid cytology to evaluate potential genetic-environmental interactions with breast cancer risk factors. For the purposes of this presentation, breast cancer risk factors are considered as being predominantly environmental in origin and of "low dose" in their magnitude.

Breast Secretory Activity and Breast Cancer Risk Factors

We have investigated the proportion of secretors associated with the major breast cancer risk factors. Earlier, we reported that "secretion" was associated with race, age, menopausal status and the genetically determined cerumen type (*15*). More extensive data from over 5,000 women support these findings and add new evidence. The findings on secretory activity are in accord with the endocrine-dependent nature of the breast gland; and we have found additional evidence for an association of genetically determined cerumen type and breast fluid secretion, evidence that mutagenic substances are secreted into the breast ducts, and that severe cytologic alterations are present in breast fluid epithelial cells from a high proportion of women with a first degree family history of breast cancer which, in turn, is associated with breast cancer risk factors.

In general, our studies of the occurrence of secretion by nipple aspiration appear to be in agreement with selected breast cancer risk factors, reflecting the degree of ovarian activity and its changes in response to reproductive history. These findings are not unexpected, as most known breast cancer risk factors are strongly associated with endocrine and reproductive characteristics.

Biochemical Aspects of Breast Fluid Secretion

Based on our studies, we have proposed that the endocrine and genetically dependent secretory activity of the breast can provide a physiologic mechanism whereby initiating and promoting chemical carcinogens can reach the breast alveolar-ductal system. In support of this hypothesis, we have found that a variety of exogenously derived substances are secreted by the nonlactating breast (*14*). We hypothesize that in genetically predisposed and metabolically activated breast epithelium the uptake and secretion of mutagenic substances conceivably could lead to the initiation, accumulation and promotion of mutant epithelial cells and eventually to the emergence of cancer cells or to benign-type breast conditions.

We have found evidence that putative mutagenic substances do reach the breast fluid (*16*). In 612 breast fluids specimens, tested with the Ames Salmonella TA 1538 strain with S-9 homogenate, we found 31 (7%) to give a positive test. The nature of the substance or substances giving the positive test is unknown. It is possible that chemical substances like hair dyes, food components, chemicals used in the workplace, substances in tobacco smoke, lipid peroxides, etc., may be responsible for positive Ames tests. It is of interest also that intravenous administration of thiotepa to a patient was followed, within a few minutes, by a positive Ames test. Moreover, two women with strongly positive Ames tests were under medication with a chlorinated phenothiozine tranquilizer (Eskatrol) shown to cause positive Ames tests. We have not been able to find any association between Ames positivity and the reproductive breast cancer risk factors.

A fundamental question can be asked: "Do these exogenous substances do anything of clinical or biological significance to the breast epithelium?" The answers may be provided by newer and more refined techniques of cell cultures of breast fluids. However, some recent findings suggest it may be possible to define, more precisely, by epidemiologic and cytologic methods, high and low risk groups of women.

Cytologic Studies of Breast Fluid: Associations with Breast Cancer Risk Factors

Breast fluid contains epithelial cells that are desquamated from the epithelial lining, the alveoli and the ducts. Nipple aspirate cytology, studied by Papanicolaou (*17*), Sartorius (*18*), King (*19, 20*), Buehring (*21*) and others, indicate that cytologic atypical hyperplasia and dysplasia are likely to reflect precursor glandular lesions and occur in association with breast cancer. Table 1 summarizes our findings in cytology from over 3800 women who underwent nipple aspiration. Of breast cancer patients, 64% had atypical hyperplasia; of those with biopsied benign breast disease 31% had atypical cells, and of clinically normal women only 15% had atypical cells, indicating a very high relative risk of atypical cytology in women with breast cancer, a somewhat lower risk in benign disease, and a smaller but still substantial proportion of risk in apparently normal women. Howev-

Table 1. Atypical cytology in nipple aspirates of breast fluid by clinical status.

Clinical status	Atypical		Relative risk	Confidence interval
	Number total	%		
Breast cancer	42/65	64.6	9.9	(6.9–14.1)[a]
Fibrocystic disease (biopsy)	77/242	31.8	2.5	(2.0–3.2)[a]
No clinical disease	552/3541	15.6	1.0	

[a]$p < 0.0001$.

er, it should be emphasized that we have not found nipple aspiration cytology to be useful as a screening device for breast cancer because of the rarity of cancer cells in breast fluid even in the presence of cancer, and the very high proportion of false positive tests that would be encountered in a screening program. In an overall tabulation by age, the proportion of cytologic atypia appears to increase from about 15% between ages 20 and 40, up to 23% by age 50 and then to decline.

Because of the strong association between atypical hyperplasia and breast cancer, we investigated possible associations of atypical hyperplasia and the major breast cancer risk factors (Table 2). Only first-degree family history of breast cancer (FH+), clinical fibrocystic disease (FCD+) and estrogen use showed a significant association with cytologic atypical hyperplasia. These observations led us to consider that nipple aspirate cytology might provide a way to investigate the association of genetic predisposition and atypical hyperplasia of breast epithelium and, presumably, susceptibility to breast cancer.

Based on these findings, we decided to examine the relationship of atypical epithelial hyperplasia and the various risk factors for breast cancer in terms of potential genetic-environmental interactions.

The model depicted in Figure 1 shows the phenotype (atypical epithelial hyperplasia) resulting from a combination of genetic susceptibility (a first-degree family history of breast cancer) and environmental factors (the other breast cancer risk factors—primarily related to the endocrine and reproductive systems) and their interaction. In this model based upon the classic "phenotype = genetics + environment," a first-degree family history of breast cancer is considered as the "genetic susceptibility factor" which may interact with a variety of "environmental" factors. The breast cancer risk factors shown in Figure 1 are considered predominantly environmentally conditioned. Studies by others (*22-24*) have attempted to examine the effect of multiple risk factors on breast cancer risk, but have not employed the genetic-environmental interaction concept presented here.

Women with a first-degree family history of breast cancer are likely to have an increased risk of developing breast cancer and are more prone if they are in the premenopausal age group. As a group, such women would be more likely to possess a gene or genes which might increase the susceptibility and responsiveness of their breast epithelium to "environmental factors." Genes could influence susceptibility to atypical hyperplasia and to breast cancer through their regulation of hormonal stimuli, or by controlling the response of breast epithelial cells to these stimuli and by influencing the metabolism of extrinsic chemical substances. We believe that these types of effects might be detectable as atypical epithelial hyperplasia in breast fluid.

The proportion of atypical cells and the relative tion," where the "genetic" susceptibility factors in

Table 2. Atypical cytology in nipple aspirates: association with breast cancer risk factors in women ≤ 50 years of age.[a]

Risk factor	At higher risk		At lower risk		Odds ratio	Confidence interval
	Atypical/total	%	Atypical/total	%		
Age menarche ≤ 12 vs. ≥ 14	113/696	16.2	23/141	16.3	.99	0.66–1.49
Nulliparous vs. parous	44/297	14.8	203/1195	17.0	0.79	0.58–1.06
1st pregnancy ≥ 31 vs. ≤ 20	13/76	17.1	37/305	12.1	1.49	0.84–2.65
Income ≥ $15,000 vs. ≤ 14,000/yr.	33/176	18.8	48/274	17.5	1.08	0.72–1.64
Oral contraceptive vs. no oral contraceptive	33/820	16.5	214/1336	16.0	1.05	0.75–1.48
Estrogen vs. no estrogen	32/151	21.1	231/1449	15.9	1.42	1.03–2.00
1° family history positive vs. negative	39/177	22.0	217/1392	15.6	1.53	1.11–2.11
Clinical FCD positive vs. FCD negative	79/406	19.4	182/1163	15.6	1.30	1.02–1.66

[a]Not age-adjusted.

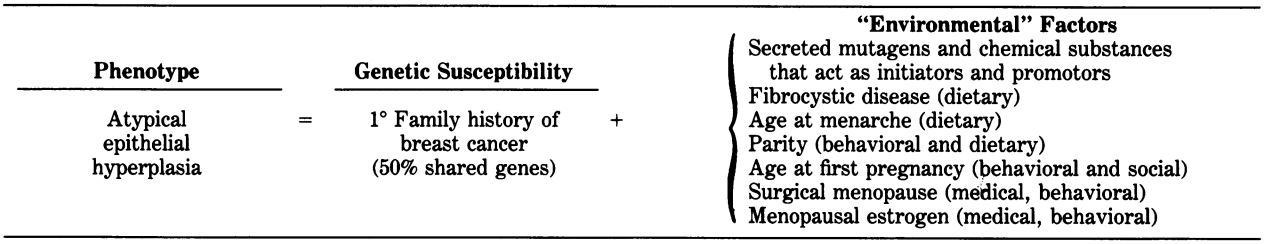

FIGURE 1. Possible genetic-environmental interactions leading to atypical breast fluid cytology. $P = G + E + GE$.

risk and confidence intervals were calculated for each combination of family history and one risk factor, using the women who are negative for both factors as the comparison group. Mantel-Haenszel summary stratification analyses were then made on the four groups. It is of interest to note that Tokuhata conducted a similar epidemiologic analysis of lung cancer mortality among subjects who had and those who did not have a family history of lung cancer and cigarette smoking (*25*). The data presented here will deal with women ≤ 50 years.

The proportion of women with atypical cytology stratified by categories of FH- and FH+ and FCD- and FCD+ are shown in Table 3. The proportion of women with atypical hyperplasia increases progressively from 14.5 to 25% and so does their relative risk. The increase would demonstrate an additive effect of family history and fibrocystic disease on the risk of atypical hyperplasia, with highest risk when both factors are positive.

The data presented above were not age-adjusted. In another article, we present age-adjusted analyses that support the conclusion of interactions similar to those in Tables 2 and 3 (*26*).

Discussion

Our findings relating breast fluid cytology and breast cancer risk factors appear to offer support for the hypothesis that women with a first-degree family history of breast cancer may have increased susceptibility to "environmental factors" as indicated by the increased proportion of atypical hyperplastic cells and an increased relative risk of atypia compared to women without a family history. The findings are also in agreement with numerous reports of the association of epithelial histologic abnormalities in "premalignant" breast cancer (*14*).

Women with both a positive first-degree family history of breast cancer and fibrocystic disease were found to have a significantly greater relative risk of atypical hyperplasia than women with only one of these factors. The presence of both factors was associated with an even higher relative risk of cytologic atypical hyperplasia in parous women, in women whose age at first pregnancy was under 20 years and in women using menopausal estrogens.

It must be emphasized that these data are from work still in progress. As noted earlier, the breast epithelia may be exposed through the breast secretory mechanism to exogenously derived chemicals, including mutagenic and carcinogenic substances. These substances might exert greater effects on "genetically susceptible" epithelia of women with both a first degree family history of breast cancer and fibrocystic disease. We suggest that our findings on breast cytology represent an example of genetic and environmental "interac-

Table 3. Proportion atypical cytology in nipple aspirates: association with family history (FH) and/or fibrocystic disease (FCD) for women ≤ 50 years of age.[a,b]

Family history	Fibrocystic disease	Atypical		Odds ratio	Confidence interval
		Number/total	%		
FH–	FCD–	151/1038	14.5	1.00	
FH–	FCD+	66/354	15.8	1.35	1.03–1.76
FH+	FCD–	26/125	20.8	1.54	1.05–2.27
FH+	FCD+	13/52	25.0	1.96	1.15–3.46

[a]Mantel-Haenszel relative risk 1.47 (1.20–1.80).
[b]Not age-adjusted.

women with a first degree family history of breast cancer may interact with "environmental factors" which potentially may lead to breast disease. The findings lead us to propose that the breast epithelium of women who have both positive first-degree family history of breast cancer and clinical fibrocystic disease may be more prone to abnormal differentiation than the breast epithelium of women without these risk factors. The biochemical mechanisms that might underlie these processes are as yet unknown. The use of first-degree family history of breast cancer as "genetic" susceptibility is clearly not completely warranted. Probably the less specific term "host" might have been more correct. However, the positive and consistent findings in combinations of risk factors with FH+ compared with FH- women suggests that our approach may be a productive way to attempt to more precisely focus on possible genetic susceptibility factors in breast cancer epidemiology.

Future studies, stratified according to family history of breast cancer and risk factors as presented here, would offer an opportunity for epidemiological and laboratory investigations of potential genetic-environmental interactions of environmental carcinogens, dietary and other factors on the production and reversal of atypical hyperplasia of breast epithelium. Control of atypical hyperplasia might lead to means of preventing breast disease and breast cancer.

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REFERENCES

1. Motulsky, A. G. Ecogenetics: genetic variation in susceptibility to environmental agents. In: Excerpta Medica International Congress, Series 411. Human Genetics. Proceedings of the Fifth International Congress of Human Genetics. Mexico City, 10-15 October 1976. Exerpta Medica, Amsterdam, pp. 375-385.
2. Hiatt, H. H., Watson, J. D., and Winsten, J. A., Eds. Origins of Human Cancer, Book B, Mechanisms of Carcinogenesis. (Cold Spring Harbor Conferences on Cell Proliferation, Vol. 4), Cold Spring Harbor Laboratory, 1977.
3. Allison, A. C. Malaria in carriers of the sickle cell trait and in newborn children. Exp. Parasitol. 68: 418-447 (1957).
4. Motulsky, A. G., and Stamatoyannopoulos, G. Clinical implications of glucose-6-phosphate dehydrogenase deficiency. Ann. Intern. Med. 65: 1329-1333 (1966).
5. Evans, D. A. P. Pharmacogenetics. Am. J. Med. 34: 639-662, 1963.
6. Miller, E. C. and Miller, J. A.: Milestones in chemical carcinogenesis. Seminars in Oncol. 6: 445-460 (1979).
7. Nebert, D. W., Robinson, J. R., Niwa, A., Kumaki, K., and Poland, A. P. Genetic expression of aryl hydrocarbon hydroxylase in the mouse. J. Cell Physiol. 85: 393-414 (1975).
8. Kellerman, G., Shaw, C. R., and Luyten-Kellerman, M. Aryl hydrocarbon hydroxylase inducibility and bronchogenic carcinoma. New Engl. J. Med. 289: 934-937 (1973).
9. Nebert, D. W., and Atlas, S. A. Aryl hydrocarbon hydroxylase induction (Ah locus) as a possible genetic marker for cancer. In: Genetics of Human Cancer. J. J. Mulvihill, R. W. Miller, and J. F. Fraumeni, Jr., Eds., Raven Press, New York, 1977, pp. 301-319.
10. Cleaver, J. E. Defective repair replication of DNA in xeroderma pigmentosum. Nature 218: 652-656 (1968).
11. Cleaver, J. E. Human diseases with *in vitro* manifestations of altered repair and replication of DNA. In: Genetics of Human Cancer, J. J. Mulvihill, R. W. Miller, and J. F. Fraumeni, Jr., Eds., Raven Press, New York, 1977.
12. Petrakis, N. L. Genetic factors in the etiology of breast cancer. Cancer 39: 2709-2715 (1977).
13. King, M.-C., Go, R. C. P., Elston, R. C., Lynch, H. T., and Petrakis, N. L. Allele increasing susceptibility to breast cancer may be linked to the glutamate-pyruvate transaminase locus. Science 208: 406-408 (1980).
14. Petrakis, N. L. Breast secretory activity in nonlactating women, postpartum breast involution, and the epidemiology of breast cancer. Natl. Cancer Inst. Monogr. 47: 161-164 (1977).
15. Petrakis, N. L., Mason, L., Lee, R. E., Sugimoto, B., Pawson, S., and Catchpool, F. Association of race, age, menopausal status, and cerumen type with breast fluid secretion in nonlactating women as determined by nipple aspiration. J. Natl. Cancer Inst. 54: 829-834 (1975).
16. Petrakis, N. L., Maack, C. A., Lee, R. E., and Lyon, M. Mutagenic activity in nipple aspirates of human breast fluid. Cancer Res. 40: 188-189 (1980).
17. Papanicolaou, G. N., Holmquist, D. G., Bader, G. M., and Falk, E. A. Exfoliative cytology of the human mammary gland and its value in the diagnosis of cancer and other diseases of the breast. Cancer 11: 377-409 (1958).
18. Sartorius, O. W., Smith, H. S., Morris, P., Benedict, D., and Friesen, L. Cytological evaluation of breast fluid in the detection of breast diseases. J. Natl. Cancer Inst. 59: 1073-1080 (1977).
19. King, E. B., Barrett, D., King, M.-C., and Petrakis, N. L. Cellular composition of the nipple aspirate specimen of breast fluid. I. The benign cells. Am. J. Clin. Pathol. 64: 728-738 (1975).
20. King, E. B., Barrett, D., and Petrakis, N. L. Cellular composition of the nipple aspirate specimen of breast fluid. II. Abnormal findings. Am. J. Clin. Pathol. 64: 739-748 (1975).
21. Buehring, G. C. Screening for breast atypias using exfoliative cytology. Cancer 43: 1788-1799 (1979).
22. Shapiro, S., Goldberg, J.,Venet, L., and Strax, P. Risk factors in breast cancer—a prospective study. In: Host-Environment Interactions in the Etiology of Cancer in Man, R. Doll and I. Vodopija, Eds., IARC, Lyon, 1973, pp. 169-182.
23. Farewell, V. T. The combined effect of breast cancer risk factors. Cancer 40: 931-936 (1977).
24. Soini, I., and Hakama, M. Failure of selective screening for breast cancer by combining risk factors. Int. J. Cancer 22: 275-281 (1978).
25. Tokuhata, G. K. Cancer of the lung: host and environmental interaction. In: Cancer Genetics, H. T. Lynch, Ed., C. C Thomas, Springfield, Ill., 1976, pp. 213-232.
26. Petrakis, N. L., Ernster, V. L., King, E. B., and Sacks, S. T. Epithelial displasia in nipple aspirates of breast fluid: association with family history and other breast cancer factors. J. Natl. Cancer Inst., in press.